Claims

1. A method for determining in a sample the functional activity of a blood coagulation component, the said activity of which can be related to the conversion of a substrate specific for activated Protein C (APC); said method being an assay for components involved in the Protein C anticoagulant system, Protein C, activated Protein C, Protein S or anticoagulant Factor V being assayed, said method comprising measuring in an assay medium, containing the sample and a substrate for APC, the conversion of the substrate caused by APC and correlating the measured value in a known manner to the activity of the component to be determined; in which method, optionally, one or two, preferably two, substances are added to the assay medium, said substance(s) being selected from APC, Protein S or an inhibitor that blocks sample derived Protein S activity, and Factor V, having anticoagulant activity or an inhibitor that blocks the same sample derived activity; with the proviso that one of the remaining substances, i.e. APC, Protein S and Factor V having anticoagulant activity is present in the sample and is the component, the functional activity of which is to be determined, for Factor V, the said activity being anticoagulant activity as cofactor to APC.

2. The method of claim 1, wherein the anticoagulant activity of Factor V as a cofactor to APC is determined, optionally in the presence of added Protein S, or an inhibitor that blocks sample derived Protein S activity.

3. The method of claim 1, wherein Protein C, after activation to APC, or APC is determined, at least one of Factor V, having APC-cofactor activity or an inhibitor that blocks the same sample derived activity, and Protein S or an inhibitor that blocks sample derived Protein S activity being added.

4. The method of claim 1, wherein Protein S is determined, at least one of Factor V having APC cofactor activity or an inhibitor that blocks the same sample derived activity, and APC being added.

5. The method of any of claims 1-4, wherein Factor VIII and/or $VIII_a$ is added to the assay medium.

6. The method of claim 1, wherein the anticoagulant activity of Factor V as a cofactor to APC is determined, optionally in the presence of added Protein S

or the said Protein S inhibitor, the substrate for APC is comprised of Factor V_a and/or Factor $VIII_a$ and the sample is derived from an individual, coagulation proteins of the sample being utilized to measure APC substrate conversion; with the proviso that when the sample is derived from an individual on therapy with vitamin K antagonists or otherwise deficient in vitamin K dependent coagulation factors, the activities of such vitamin K dependent factors in the sample are modified by addition of at least one vitamin K dependent coagulation factor in activated or unactivated form, optionally in combination with Protein S.

7. The method according to any of claims 1-6, wherein the functional level of each selected substance added to the assay medium is essentially constant in the assay media of samples to be compared.

8. The method according to claim 7, wherein the essentially constant level is achieved by including into the assay medium a functional excess of the selected substance compared to the level provided by the sample.

9. The method of claim 7, wherein as the inhibitor, an antibody is used binding specifically to an epitope that is associated with the activity of APC, Protein C, or Protein S, or anticoagulant activity of Factor V as cofactor to APC.

10. The method according to any of claims 1-9, wherein the sample is a blood or blood derived sample, such as a plasma sample.

11. The method according to any of claims 1-10, wherein the one or two selected substance(s) has/have been provided in form of plasma deficient in the substance to be assayed.

12. The method according to any of claims 1-11, wherein the found level of the substance assayed is used to diagnose a blood coagulation disorder in the individual from which the sample is derived.

13. A method for diagnosing a blood coagulation/anticoagulation disorder, preferably a thromboembolic disorder, or for determining predisposition therefor, in an individual, preferably a mammal, such as a human being, said method comprising determining the level of a blood component expressing anticoagulant activity in a sample, preferably a blood or blood derived sample, such as plasma, derived from said individual, said blood component being comprised of Factor V,

an abnormal level indicating manifestation of, or predisposition for, said disorder, for a decreased level said disorder preferably being a thromboembolic disorder.

14. The method of claim 13, wherein the level of anticoagulant activity of Factor V as a cofactor to APC is determined.

15. The method of claim 14, wherein the anticoagulant activity is measured in accordance with the method of claim 2 adapted for determining anticoagulant activity of Factor V as cofactor to APC.

16. The method of claim 13, wherein Factor V having anticoagulant activity as a cofactor to APC is determined with an immunological method.

17. The method of claim 14, wherein the anticoagulant activity of Factor V as a cofactor to APC is determined in accordance with the method of claim 6.

18. The method of claim 15, wherein the anticoagulant activity of Factor V as a cofactor to APC is determined in the sample based on coagulation analysis, suitably with the method of claim 2, (i) one portion of the sample being incubated in absence of added APC but, optionally, in presence of further blood coagulation components required to enable measurement of substrate conversion by APC, and (ii) one portion of the sample being incubated in presence of added APC and, optionally, in presence of further blood coagulation components required to enable measurement of substrate conversion by APC; the clotting time being used, optionally after suitable conversion, and results below an established cut-off value based on clotting times, obtained in the same procedure for normal individuals, being indicative of a deficiency in anticoagulant activity of Factor V as cofactor to APC.

19. An antibody preparation reacting specifically with a region or site of Factor V that may carry an epitope associated with its anticoagulant activity as cofactor to APC.

20. The antibody preparation of claim 19, said preparation being monoclonal and comprising a definite number e.g. a number selected in the range of 1-5, of monoclonal antibodies having the specificity defined in claim 19.

21. Antibody preparation comprising polyclonal antibodies that recognize and selectively bind to Factor V, preferably to a region or site of Factor V

associated with its anticoagulant activity as cofactor to APC, said region or site optionally comprising an epitope for said activity.

22. The antibody preparation of claim 20 comprising monoclonal antibodies, said antibodies being produced by mouse/mouse hybridoma cells, and preferably produced by the hybridoma cell line deposited in the European Collection of Animal Cell Culture under the provisional accession number AM-4-5-1 93120846.

23. A cell line producing [monoclonal] antibodies reacting specifically with a region or site of Factor V that may carry an epitope associated with its anticoagulant activity as cofactor to APC.

24. The cell line of claim 23, which is a hybridoma cell line.

25. The cell line of claim 24, which is the hybridoma cell line deposited in the European Collection of Animal Cell Culture under the provisional accession number AM-4-5-1 93120846.

26. Use of Factor V, subunits or fragments thereof, having anticoagulant activity as a cofactor to APC for the manufacture of a medicament or pharmaceutical preparation for enhancing, or restoring to normal, in an individual the anticoagulant activity of Protein C, APC, Factor V or Protein S, or any combination thereof.

27. Use according to claim 26, a medicament or pharmaceutical preparation for treatment of vascular diseases, preferably thromboembolic disorders, such as thrombosis and disseminated intravascular coagulation, being manufactured.

28. A plasma package preparation intended for the determination in vitro of anticoagulant activity of Factor V, as cofactor to APC, said preparation being comprised of human plasma, which has been made deficient in said anticoagulant activity, e.g. by immune depletion with respect to Factor V, capable of expressing said activity, said plasma optionally being supplemented with Factor V, e.g. bovine Factor V, from a species inherently lacking said anticoagulant activity, or with Factor V_a or said preparation being comprised of human plasma from one or more individuals, whose plasma is deficient in said activity.

29. The plasma package preparation of claim 28, wherein the deficient plasma is mixed with normal plasma, or with Factor V having anticoagulant

activity, the mixture being capable to express the said anticoagulant activity at a suitable low level, e.g. for use as control plasma.

30. A plasma package preparation intended for determination in vitro of anticoagulant activity of Factor V as cofactor to APC, said preparation being a mixture of plasma from individuals having partial deficiency in Factor V anticoagulant activity, said mixture being capable to express said anticoagulant activity at a suitable low level, e.g. for use as control plasma.

31. A plasma deficient in anticoagulant activity of Factor V as cofactor to APC, said plasma being comprised of human plasma, which has been made deficient in said anticoagulant activity, e.g. by immune depletion with respect to Factor V capable of expressing said activity, said plasma optionally being supplemented with Factor V, e.g. bovine Factor V, from species inherently lacking said anticoagulant activity, or with Factor V_a, or being comprised of human plasma from one or more individuals, whose plasma is deficient in said activity.

32. The plasma of claim 31, wherein the deficient plasma is mixed with normal plasma, or with Factor V having said activity, or is a mixture of plasma from individuals having partial deficiency in Factor V anticoagulant activity, said mixture being capable to express said anticoagulant activity at a suitable low level, e.g. for use as control plasma.

33. Use of the antibody preparation of any of claims 19-22 to obtain an immune-depleted plasma package preparation of claim 28 or plasma of claim 31.

34. A Protein S preparation intended for the determination of anticoagulant activity of Factor V as cofactor to APC.

35. A Protein C preparation, optionally in activated form or combined with an activator for Protein C, intended for the determination of anticoagulant activity of Factor V as cofactor to APC.

36. A Protein C preparation according to claim 35, said preparation being combined with at least one vitamin K dependent coagulation factor selected from Factors VII, IX, X and II, optionally combined with Protein S.

37. Use of Protein C/activated Protein C or Protein S for the manufacture of a pharmaceutical composition intended for the treatment of a disorder related to

functional disturbances in levels of anticoagulant activity of Factor V as cofactor to APC.

38. Factor V, suitably human Factor V, capable of becoming activated to exert Factor V_a procoagulant activity but not capable of exerting anticoagulant activity, preferentially not anticoagulant activity as a cofactor to APC, said factor being in a substantially pure form.

39. Factor V, suitably human Factor V, capable of exerting anticoagulant activity, preferentially as a cofactor to APC, but not capable of expressing procoagulant activity of Factor V_a.

AMENDED CLAIMS
[received by the International Bureau on 27 June 1994 (27.06.94); new claims 40-43 added (1 page)]

functional disturbances in levels of anticoagulant activity of Factor V as cofactor to APC.

38. Factor V, suitably human Factor V, capable of becoming activated to exert Factor V_a procoagulant activity but not capable of exerting anticoagulant activity, preferentially not anticoagulant activity as a cofactor to APC, said factor being in a substantially pure form.

39. Factor V, suitably human Factor V, capable of exerting anticoagulant activity, preferentially as a cofactor to APC, but not capable of expressing procoagulant activity of Factor V_a.

40. Method to determine for an individual a predisposition to develop thrombosis due to inherited APC-resistance caused by gene mutation(s), said method comprising determining for a cell sample from said individual occurence of Factor V gene mutation(s), which mutation(s) is (are) located in one or more nucleic acid fragment(s) and/or sequences of the Factor V gene, said mutations giving rise to expression of a mutated Factor V/Va molecule, which is associated with expression of APC-resistance and, thus, predisposition to develop thrombosis.

41. Method of claim 40, wherein the said mutation(s) is (are) determined as an abnormal absence or presence of nucleic acid fragment(s) and/or sequence(s) in the Factor V gene caused by the said mutation(s), current methods, such as methods based on nucleic acid hybridization assays, nucleic acid sequencing, or immunoassays, being used.

42. Method of claim 40, wherein the said mutation(s) is (are) determined indirectly based on linkage thereof to a neutral polymorphism in the Factor V gene.

43. A method for determining in a sample, preferably a blood or blood derived sample, such as plasma, the level of a blood component expressing anticoagulant activity, wherein said blood component is comprised of Factor V and Factor V is determined with an immunological method.